

## **Supplemental material**

### **Lung histopathologic clusters in severe COVID-19: a link between clinical picture and tissue damage**

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**List of clinical-laboratory variables considered in the statistical analysis:**

1. Age; gender; body mass index; history of diabetes, respiratory disease, smoking (active or past), cardiovascular disease, cancer, chronic immunosuppressive therapy, and HIV infection; interval between symptoms onset and hospitalization; interval between symptoms onset and dyspnea onset; length of stay in hospital (LOS-H); length of stay in ICU (LOS-ICU); days on continuous positive airway pressure (CPAP); days on mechanical ventilation; interval between CPAP and mechanical ventilation onset; days on positive pressure ventilation (PPV); interval between symptoms onset and mechanical ventilation; highest positive end-expiratory pressure (PEEP) in the first 48 hours since positive pressure ventilation onset; mean PEEP in the first seven days on positive pressure ventilation (mPEEP<sub>7D</sub>); mean PEEP during whole positive pressure ventilation treatment (mPEEP<sub>TOT</sub>); cumulative PEEP over positive pressure ventilation days (cPEEP<sub>PPV</sub>); cumulative PEEP over mechanical ventilation days (cPEEP<sub>MV</sub>); tracheostomy (yes/not); need for continuous renal replacement treatment (CRRT); interval between CPAP and CRRT onset; interval between mechanical ventilation and CRRT onset; treatment with Tocilizumab, Remdesivir, Hydroxychloroquine, and lopinavir/ritonavir (yes/not); need for prone position (yes/not) during hospital stay; sepsis (yes/not) during hospital stay; worst serum albumin during hospital stay; worst D-dimer during hospital stay; kidney injury during hospitalization assessed by KDIGO classification.
2. Worst value within 48 hours since internal medicine ward or ICU admission of the following variables: Daily urinary output; daily fluid balance; Glasgow Coma Score, ratio of oxygen arterial pressure to fraction of inspired oxygen ( $paO_2/FiO_2$ ) and the corresponding values of pH,  $pO_2$ ,  $pCO_2$ ,  $SatO_2\%$ , plasma lactate; chest-X-ray score (assessed through Brixia score); airway peak pressure (in mechanically ventilated patients); tidal volume (in mechanically ventilated patients); respiratory rate; mean arterial pressure; need for norepinephrine, epinephrine or dobutamine infusion (and dosages); serum creatinine; white blood cell count (total and neutrophils and lymphocytes count); neutrophils to lymphocytes ratio; haemoglobin; haematocrit; platelets count; C-reactive protein;

procalcitonin; serum albumin; D-dimer; serum blood urea; serum bilirubin; serum aspartate-amino-transferase; serum alanine-amino transferase; serum lactate-dehydrogenase; serum cholesterol (total and HDL and LDL partitions); serum phosphate; serum magnesium, calcium, sodium, potassium and chloride; sequential organ failure assessment (SOFA) score. First available value of interleukin-6 and ferritin within one week since hospital admission.

e-Table 1

Clinical-biochemical-radiological characteristics of patients included in the final data analysis.

	N=48
<b>Age, years</b>	64.5 (60-71)
<b>BMI</b>	27.7 (24.5-31.1)
<b>Gender, M/F, n (%)</b>	44/4 (91.7/8.3)
<b>Comorbidities, n (%)</b>	
Smoke	9 (18.8)
Cardiovascular disease	30 (62.5)
Respiratory disease	3 (6.3)
Immunosuppressive therapy	2 (4.2)
HIV	1 (2.1)
Diabetes	5 (10.4)
Cancer	6 (12.5)
<b>Symptoms onset-to-hospital admission interval, days</b>	7 (4-10)
<b>Symptoms onset-to-mechanical ventilation, days</b>	12 (8.7-17.5)
<b>Chest-X-ray score<sup>a</sup></b>	15 (13-16)
<b>CPAP treatment, n</b>	39 (81.2)
<b>CPAP days</b>	2 (1-5)
<b>Patients treated with MV, n</b>	43 (89.6)
<b>MV days</b>	15 (7-19)
<b>PPV days</b>	17.5 (9-23.7)
<b>Respiratory Rate, bpm</b>	20 (18-24)
<b>FiO<sub>2</sub></b>	0.8 (0.7-0.8)
<b>PEEP, cmH<sub>2</sub>O</b>	14 (12-17.5)
<b>mPEEP<sub>7D</sub>, cmH<sub>2</sub>O</b>	14 (11.4-16.6)
<b>mPEEP<sub>TOT</sub>, cmH<sub>2</sub>O</b>	13.6 (10.5-16)
<b>cPEEP<sub>PPV</sub>, cmH<sub>2</sub>O·days</b>	193.4 (123.4-279.5)
<b>Ppeak, cmH<sub>2</sub>O</b>	33 (30-35)
<b>Tidal Volume, ml</b>	550 (500-600)
<b>GCS</b>	7 (3-15)
<b>PaO<sub>2</sub>/FiO<sub>2</sub> ratio</b>	116.5 (93.7-142.5)
<b>ABG</b>	
pH	7.33 (7.27-7.4)
pCO <sub>2</sub> , mmHg	46.5 (40-54.5)
pO <sub>2</sub> , mmHg	86.5 (70.5-101.2)
Sat, %	98 (94.7-99)
<b>Prone position, n</b>	
yes	10 (20.8)
missing	6 (12.5)
<b>Mean arterial pressure, mmHg</b>	75 (70-80)
<b>Catecholamines</b>	
Epinephrine, n (%)	1 (0.2)
Epinephrine, mean µg·Kg·min <sup>-1</sup>	0.04
Norepinephrine, n (%)	31 (64.6)
Norepinephrine, mean µg·Kg·min <sup>-1</sup>	0.03 (0.09-0.14)
Dobutamine, n (%)	0
<b>Urinary output at day 1, ml</b>	930 (393-1480)
<b>Fluid balance at day 1, ml</b>	1025 (177-1817)
<b>WBC, cells·mm<sup>3</sup></b>	8545 (6467-12235)
<b>AKI, n (%)</b>	

None	11 (22.9)
KDIGO class I	11 (22.9)
KDIGO class II	4 (8.3)
KDIGO class III	22 (45.8)
<b>CRRT, n (%)</b>	11 (22.9)
<b>Neutrophils, cells·mm<sup>3</sup></b>	8340 (5060-11000)
<b>Lymphocytes, cells·mm<sup>3</sup></b>	536 (413-815)
<b>Neutrophils-to-lymphocytes ratio</b>	16.3 (7.7-23.1)
<b>Hemoglobin, g·dl<sup>-1</sup></b>	12.7 (11.5-13.8)
<b>Hematocrit, %</b>	37.5 (35-41)
<b>Platelets, 10<sup>3</sup> cells·mm<sup>3</sup></b>	217.5 (174-331)
<b>C-reactive protein, mg·l<sup>-1</sup></b>	220 (126-322)
<b>Procalcitonin, µg·l<sup>-1</sup></b>	1.25 (0.27-2.6)
<b>S-albumin, g·dl<sup>-1</sup></b>	2.3 (2-2.7)
<b>Worst S-albumin, g·dl<sup>-1</sup></b>	1.7 (1.6-2)
<b>D-dimer, ng·ml<sup>-1</sup></b>	3766 (1358-13396)
<b>Worst D-dimer, ng·ml<sup>-1</sup></b>	13390 (3758-29524)
<b>Fibrinogen, mg·ml<sup>-1</sup></b>	700 (695-700)
<b>IL-6, ng·l<sup>-1</sup></b>	254 (160-1047)
<b>Ferritin, µg·l<sup>-1</sup></b>	2560 (1282-3494)
<b>S-creatinine, mg·dl<sup>-1</sup></b>	0.95 (0.78-1.41)
<b>S-urea, mg·dl<sup>-1</sup></b>	53.5 (33.7-83.7)
<b>Bilirubin, mg·dl<sup>-1</sup></b>	1.2 (1.2-1.35)
<b>AST, U·l<sup>-1</sup></b>	60.5 (42-100.7)
<b>ALT, U·l<sup>-1</sup></b>	46 (34-72.7)
<b>LDH, U·l<sup>-1</sup></b>	543 (437.5-703.7)
<b>Cholesterol<sub>TOT</sub>, mg·dl<sup>-1</sup></b>	120 (98-144)
<b>HDL, mg·dl<sup>-1</sup></b>	19 (13-25)
<b>SOFA, score</b>	10 (8-11.75)
SOFA neurologic	3 (0-4)
SOFA respiratory	3 (3-4)
SOFA cardiovascular	3 (0-4)
SOFA kidney	0 (0-1)
SOFA liver	0 (0-0.75)
SOFA coagulation	0 (0-0.75)
<b>Pharmacological treatment, n (%)</b>	
Tocilizumab	12 (25)
Remdesivir	11 (22.9)
Hydroxychloroquine	31 (64.6)
Lopinavir/ritonavir	26 (54.2)
Steroids	14 (29.2)
<b>Sepsis/septic shock, n (%)</b>	28 (58.3)
<b>Microbiological isolates, n (%)</b>	
Enterococcus Faecium	12 (25)
Enterococcus Faecalis	12 (25)
Klebsiella Pneumoniae	5 (10.4)
Pseudomonas Aeruginosa	3 (6.3)
Staphylococcus coag. negative	5 (10.4)
Streptococcus spp.	1 (2.1)
Candida spp.	0
Serratia Marcescens	0
Enterobacter aerogenes	3 (6.3)

Staphylococcus Aureus	2 (4.2)
<b>LOS-ICU, days</b>	<b>15 (6.25-19.75)</b>
<b>LOS-Hospital, days</b>	<b>17.5 (11-25)</b>

Data are shown as median (IQR) or n (%) were indicated.

<sup>a</sup> Chest-X-ray score according to Brixia classification. BMI, body mass index; CPAP, continuous positive airway pressure; MV, mechanical ventilation; PPV, positive pressure ventilation; PEEP, positive end expiratory pressure; mPEEP<sub>7D</sub>, mean PEEP applied in the first seven days since admission; mPEEP<sub>TOT</sub>, mean PEEP applied with positive pressure ventilation during hospitalization; cPEEP<sub>PPV</sub>, cumulative PEEP during positive pressure ventilation; Ppeak, peak airway pressure measured at admission in mechanically ventilated patients; GCS, Glasgow Coma Score; ABG, arterial blood gas analysis; WBC, white blood cells; AKI, acute kidney injury; CRRT, continuous renal replacement therapy; IL-6, plasma interleukin 6; AST, aspartate-amino transferase; ALT, alanine-amino transferase; LDH, lactic dehydrogenase; HDL, high density lipoprotein; SOFA, Sequential Organ Failure Assessment; LOS, length of stay; ICU, Intensive Care Unit.

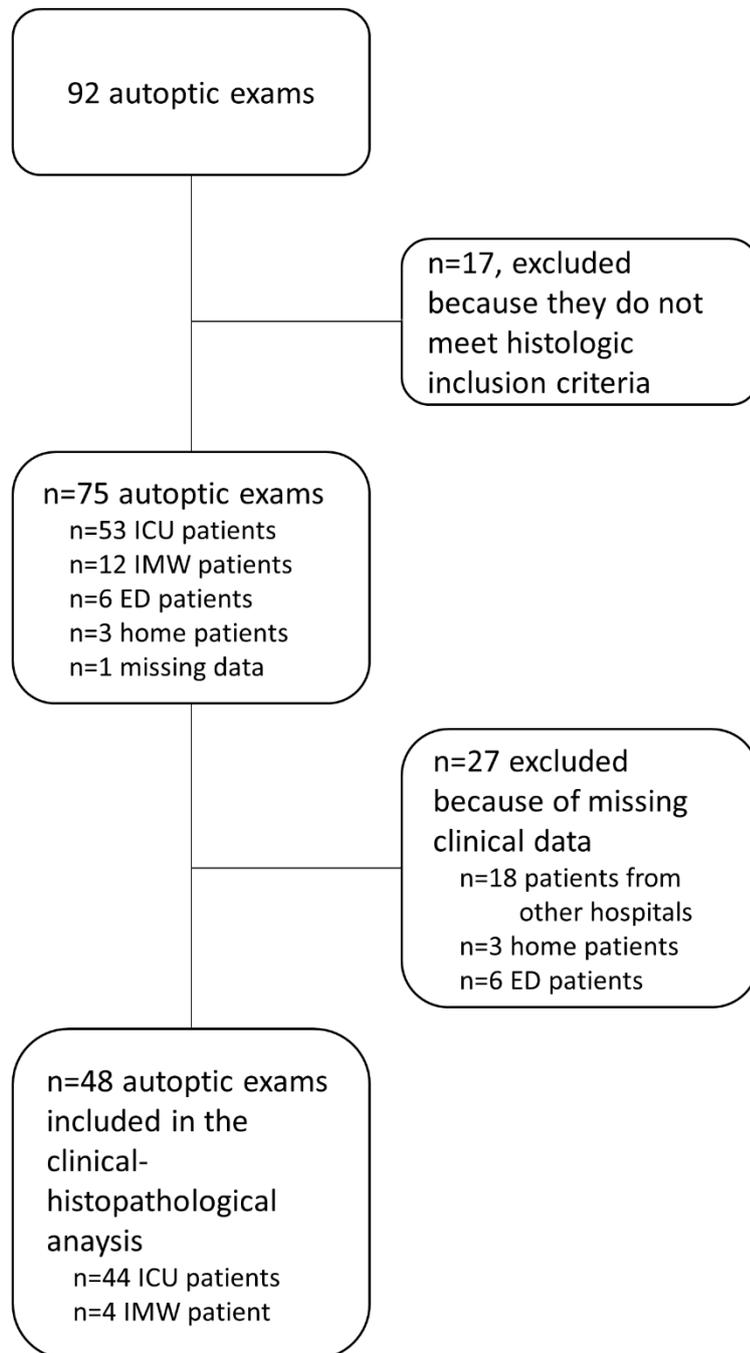
e-Table 2

Histopathologic findings in the whole cohort (n=75) and in subgroups.

	Whole cohort (n=75)	Clinical data available (n=48)	No clinical data (n=27)	p-value
<b>Histopathologic patterns</b>				
Exudative DAD	3 (1-5)	4 (1-5.75)	2 (0-5)	0.278
Early proliferative DAD	8 (2-10)	10 (7.25-10)	0 (0-5)	<0.0001
AFOP	0 (0-1)	0 (0-1)	0 (0-0)	0.006
Interstitial pneumonia	5 (3-5)	5 (4-5.75)	4 (1-5)	0.018
Late proliferative DAD	0 (0-4)	2 (0-5)	0 (0-0)	<0.0001
Bronchopneumonia	2 (0-5)	3 (0-6)	0 (0-4)	0.03
Thrombi	3 (0-5)	3 (2-5.75)	0 (0-4)	0.02
Megakaryocytes	6 (5-10)	6 (5-10)	5 (4-7)	0.031
Normal lung areas	0 (0-5)	0 (0-0)	7 (1-10)	<0.0001
<b>Other findings, n (%)</b>				
Aspergillus	5 (6.7)	3 (6.3)	2 (7.4)	0.99
Giant cells	7 (9.3)	7 (14.6)	0	0.045
Hemorrhage	13 (17.3)	9 (18.8)	4 (14.8)	0.76
Fibrosis	5 (6.7)	3 (6.3)	2 (7.4)	0.99
Pleuritis	5 (6.7)	5 (10.4)	0	0.15
Infarct	13 (17.3)	11 (22.9)	2 (7.4)	0.11
Lympho-plasma cells	3 (4)	3 (6.3)	0	0.55
Alveolar infiltrates				
Edema	8 (10.7)	2 (4.2)	6 (22.2)	0.022

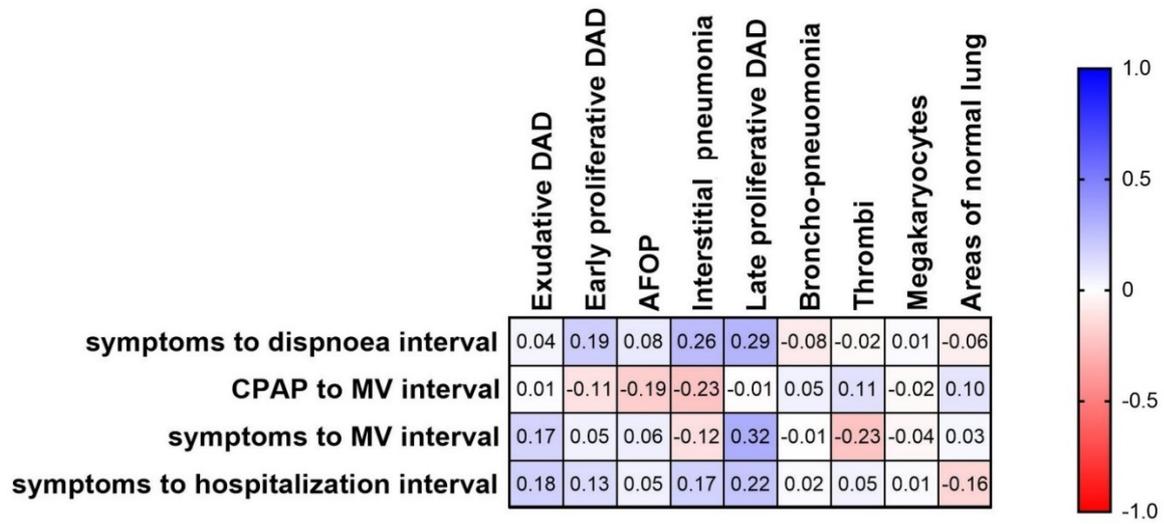
Data are shown as median (IQR) or n (%) where indicated. P-values were calculated by Mann-Whitney test.

e-Figure 1



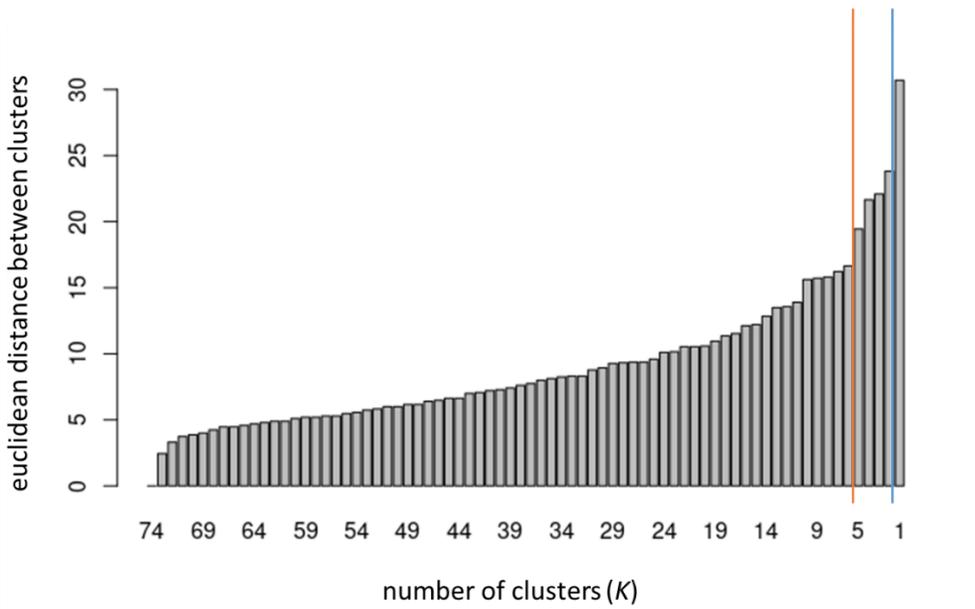
**Flow chart of the patient selection.** 92 autopsies were performed during the predefined study period. 75 had full histopathologic examination according to the inclusion criteria, 48 had also complete clinical records. *ICU*, intensive care unit; *IMW*, internal medicine ward; *ED*, emergency department.

e-Figure 2



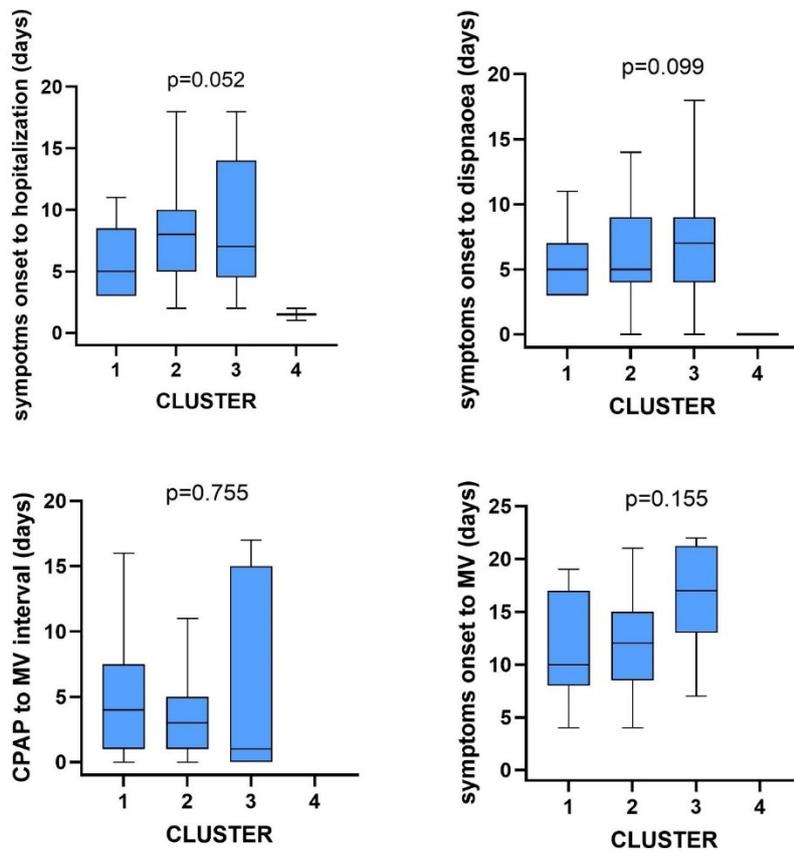
Correlation matrix (Spearman's  $r$ ) *between* intervals from i) symptoms to dispnoea, ii) CPAP to MV, iii) symptoms to MV, iv) symptoms to hospitalization *and* the histopathologic patterns. Correlation coefficients are shown into the cells and none of them was significant.

e-Figure 3



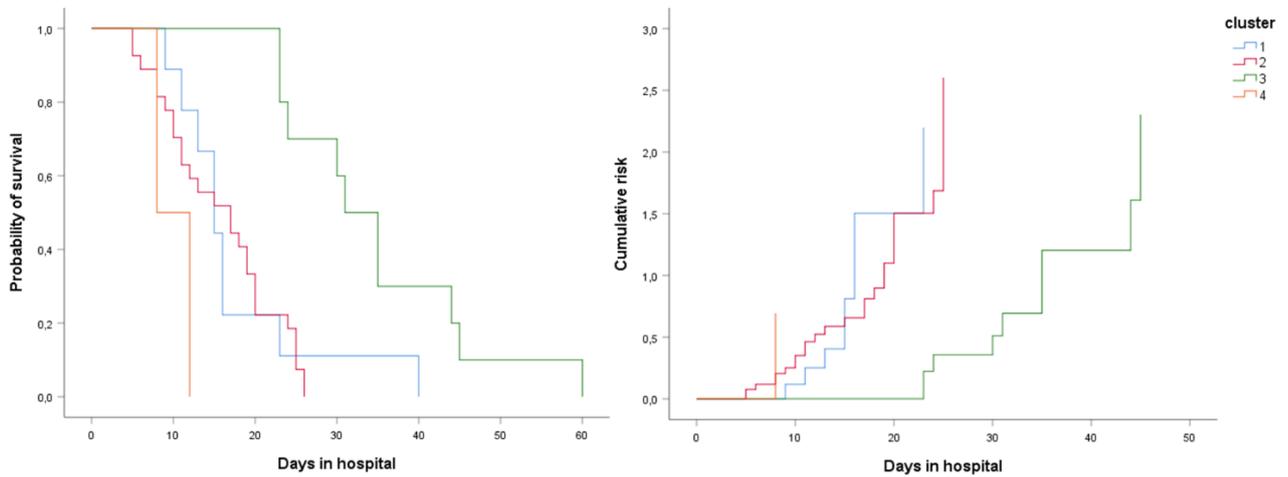
**Choice of the number of clusters (K).** According to the “elbow” method, the difference of heights plotted in the figure shows that the largest jumps of heights was between 1 and 2 clusters (blue line) and between 5 and 6 clusters (orange line). Two-cluster based analysis was able to discriminate almost all patients with clinical information from those without it. In order to characterize the histopathologic cluster, the second “elbow” was more representative.

e-Figure 4



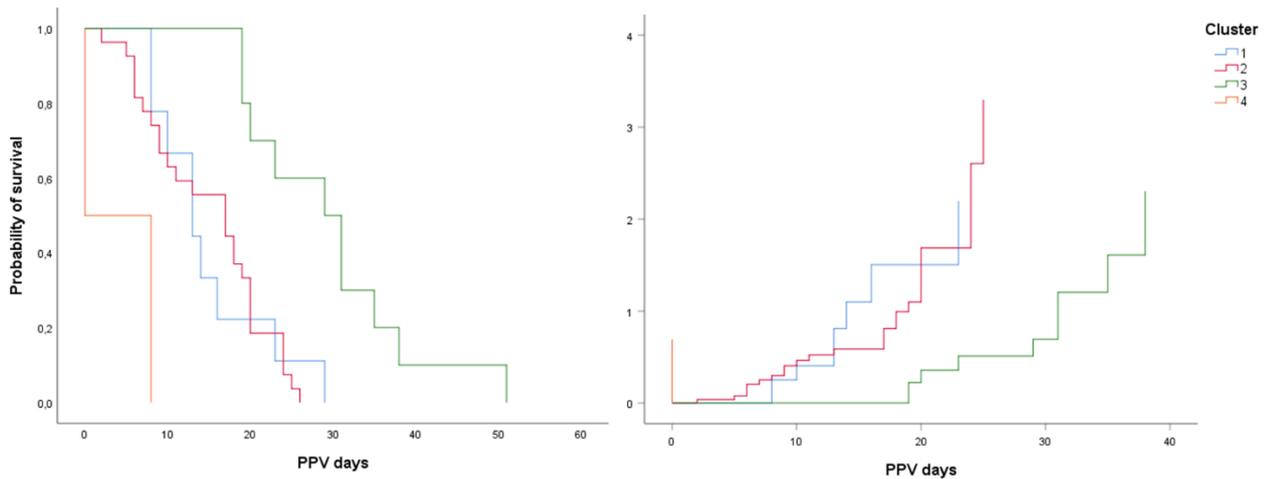
Distribution of intervals from symptoms onset to hospital admission, from symptoms to dispnoea onset, from symptoms onset to MV start, and from CPAP to MV start among clusters. P-values were calculated with Kruskal-Wallis test (see Methods in the text).

**e-Figure 5**



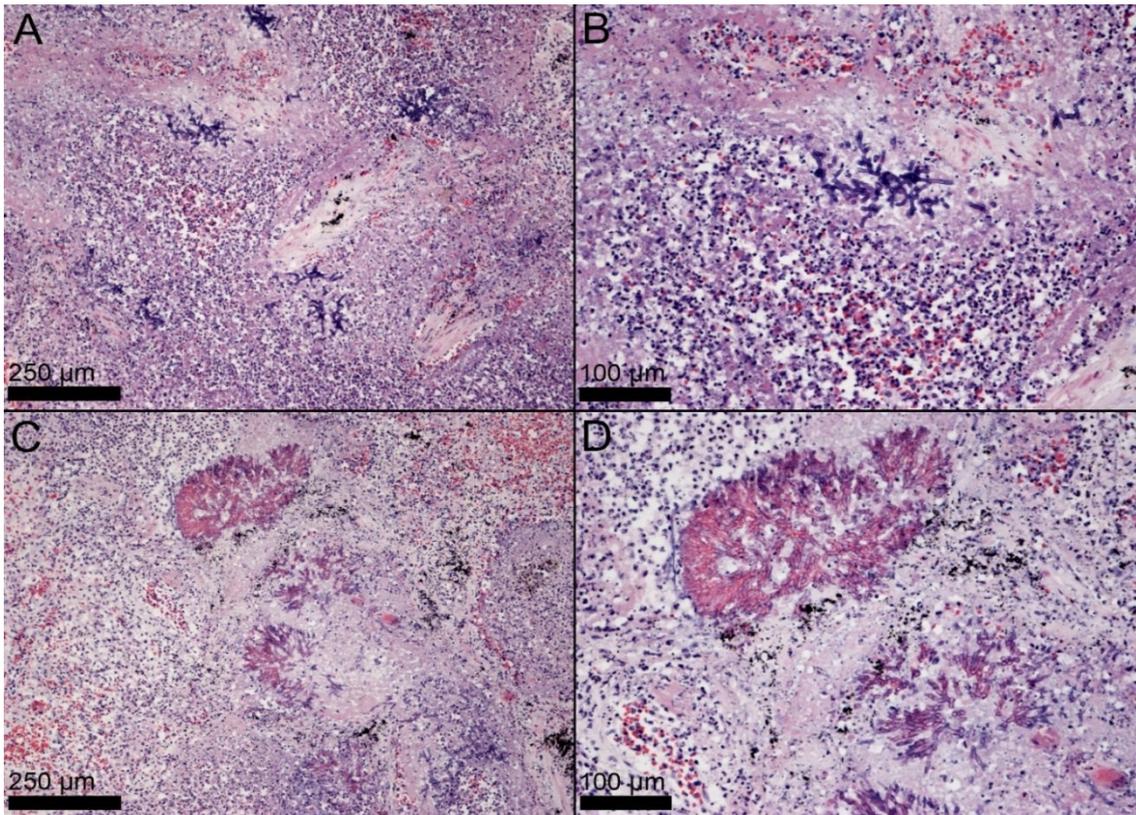
Detailed Kaplan Meier curves of clusters of patients with full clinical records as shown in Figure 6 in the text.  $p < 0.0001$  (Log Rank Mantel–Cox test).

**e-Figure 6**



Kaplan Meier curves of clusters of patients with full clinical records who underwent positive pressure ventilation (PPV)  $P < 0.0001$  (Log Rank Mantel–Cox test).

e-Figure 7



**Histopathology of a case with aspergillosis.** Areas of acute inflammatory infiltrate, cellular debris, and scattered basophilic hyphae (A, 10x, H&E; B, 20x, H&E) admixed with other areas with floriid eosinophilic hyphae (C, A, 10x, H&E; D, 20x, H&E) in case n. 8.